Light-induced photosynthetic electron transfer upon anaerobiosis in *Chlamydomonas*: Kinetics, electron sinks and setup of a fluorescence screen to identify new players

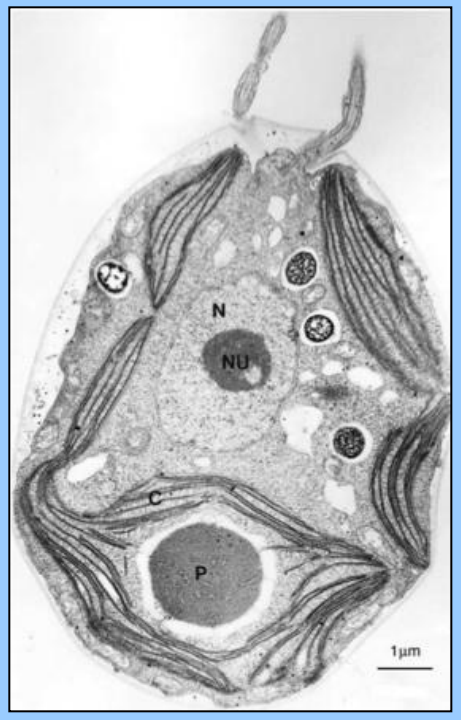
Damien Godaux[1]*, Barbara Emonds-Alt[1], Jean Alric[2], Bart Ghysels[1], Claire Remacle[1], Pierre Cardol[1]

[1]Institut de Botanique, Université de Liège, Belgique;
[2]Institut de Biologie Physico-Chimique, Université Paris VI, France

Funding: F.R.S.-FNRS
FP7 funded project Sunbiopath (grant agreement 245070)
ARC (Micro-H2)

Young Algaeneers symposium
Wageningen, The Netherlands
14 – 16 june 2012

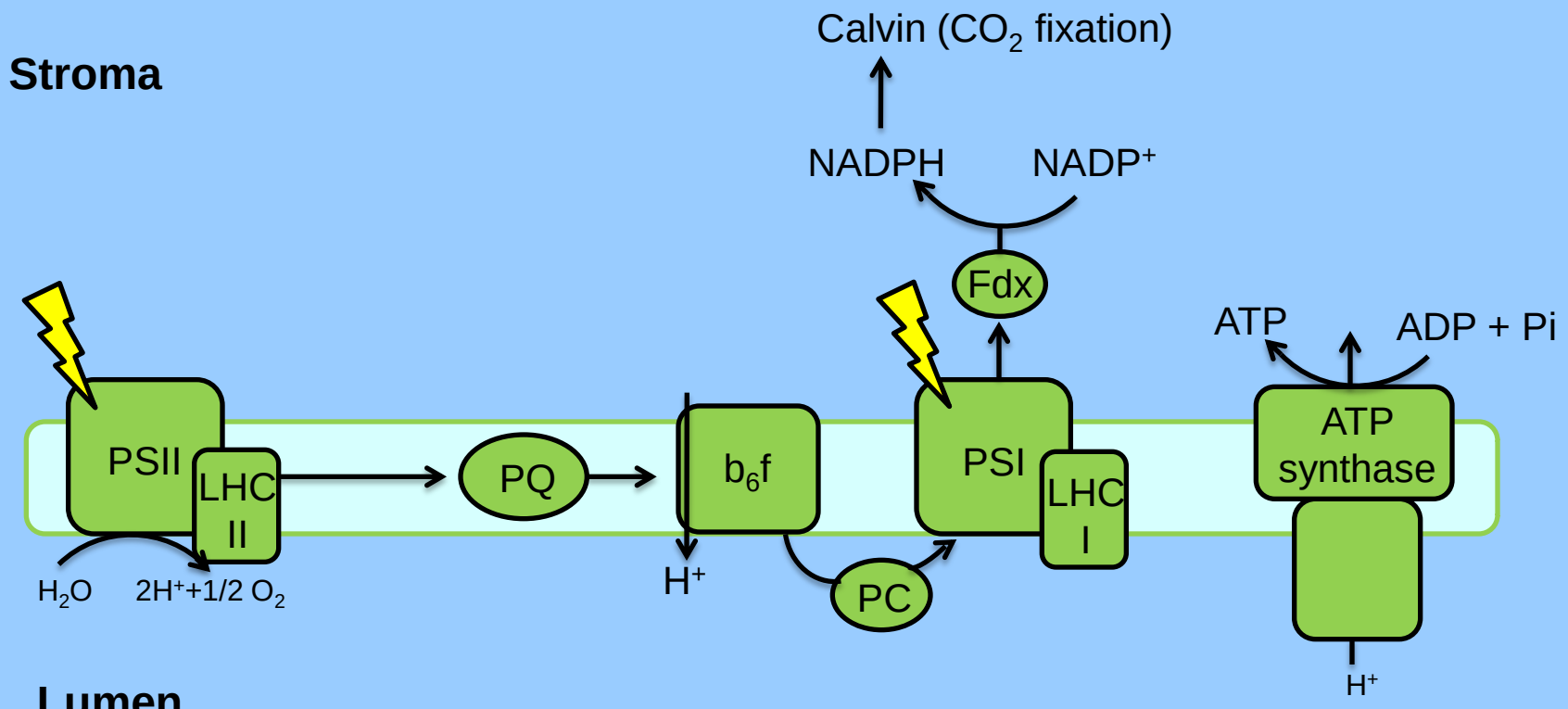
Chlamydomonas



<u>Model system for unicellular eukaryote</u>

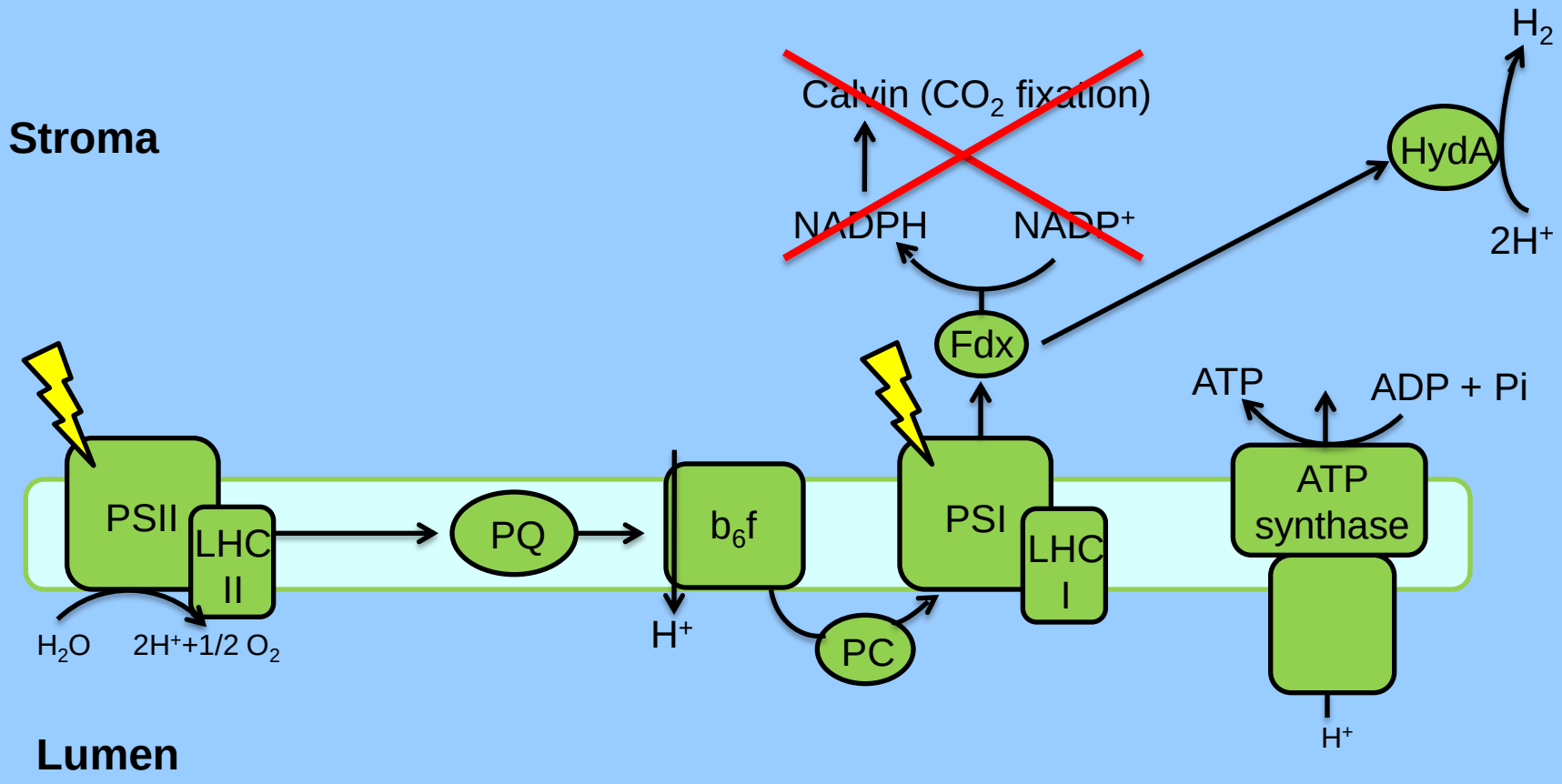
- ❑ Green algae from Chlorophyceae class
- ❑ Three genomes are completely sequenced
- ❑ Easy to transform → model for genetics studies
- ❑ Life cycle (sexual et asexual) easy to control in laboratory
- ❑ Heterotrophic growth (exogenous carbon source)
- ❑ phototrophic growth (photosynthetic assimilation of CO_2)
- ❑ mixotrophic growth (combination of both)
- ❑ Model for flagella assembly, respiration (mitochondria) and photosynthetic (chloroplast) studies

Photosynthetic electron transfer pathway

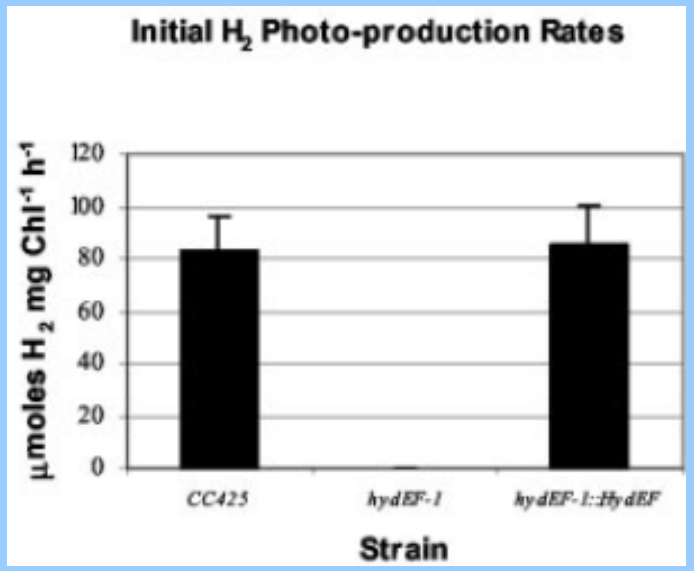


Alternative photosynthetic pathway

- Anaerobiosis hydrogenase activity



→ HydEF and HydG are two proteins required for the maturation of an active hydrogenase in *Chlamydomonas reinhardtii*



Posewitz *et al.*, (2004).
The Journal of Biological Chemistry.

http://www.phytozome.org

Wecker *et al.*, (2011).
International Journal Of Hydrogen Energy.

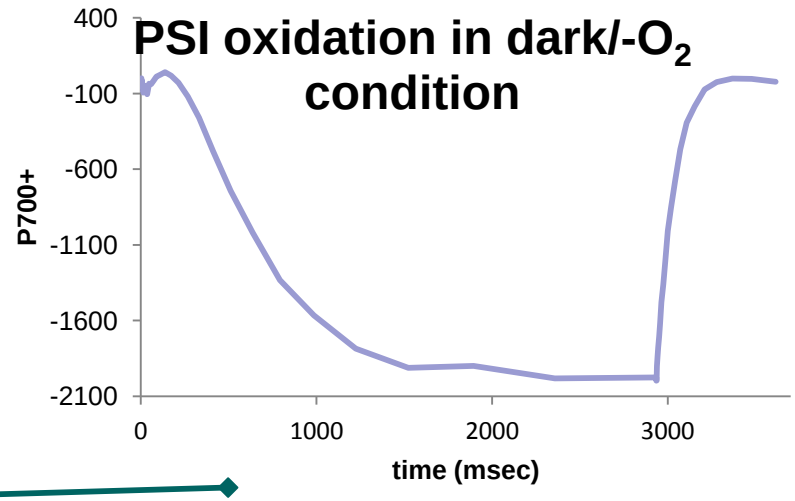
Aims of the work

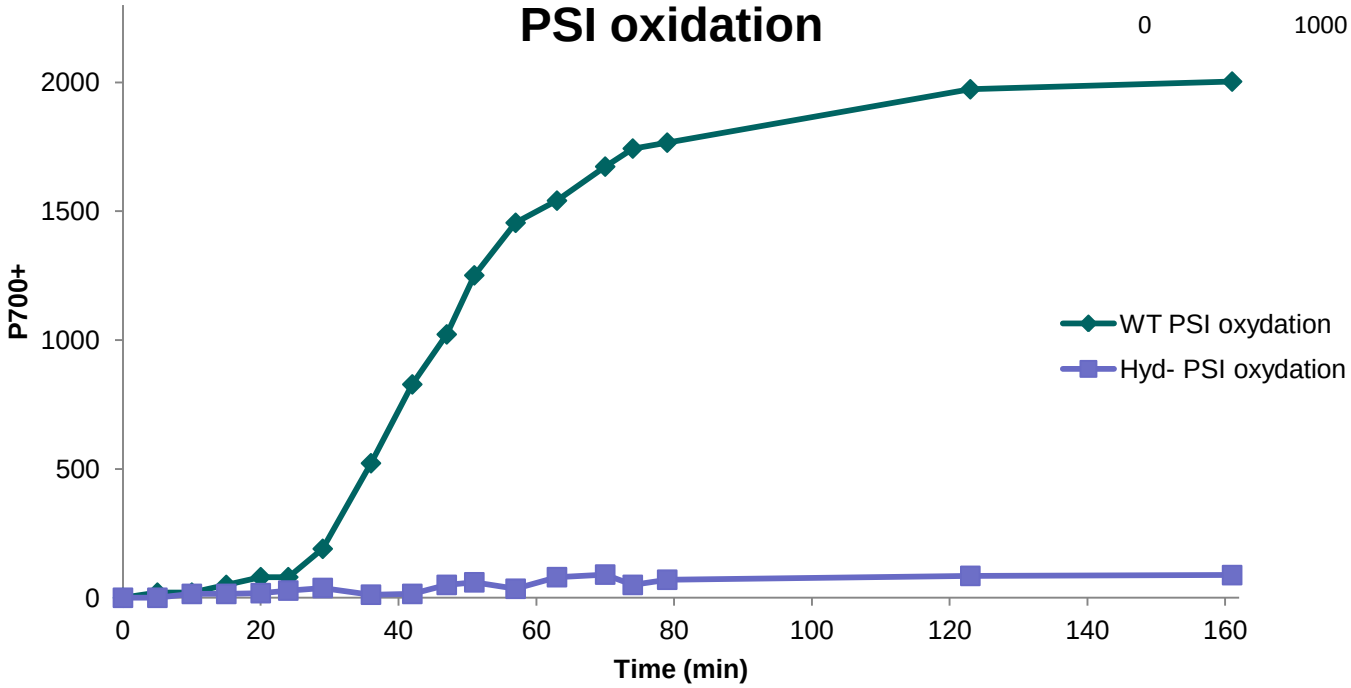
- **Study of the light-induced photosynthesis upon anaerobiosis**

  The dark/-O_2 to light transition: focus on hydrogenase (WT vs Hyd^-)

- **Application to identify new players**

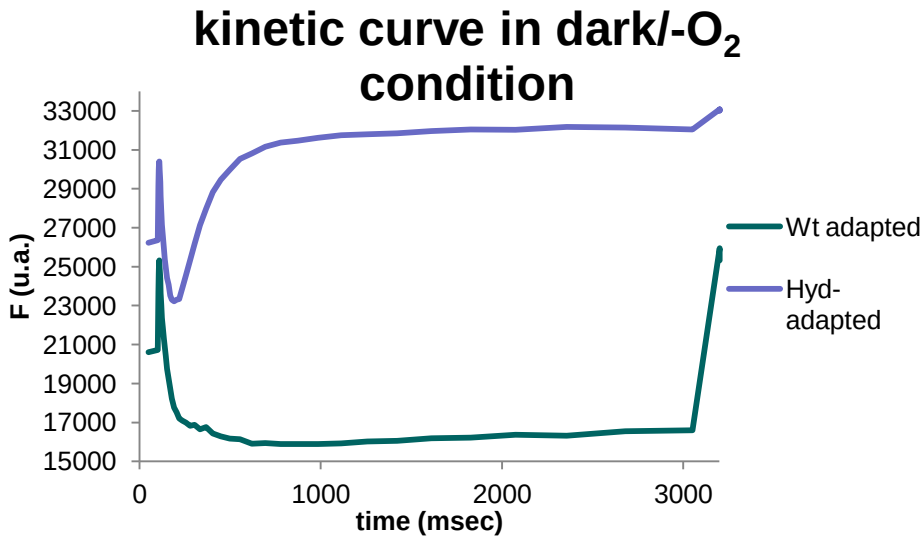
PSI activity (P700 oxidation)

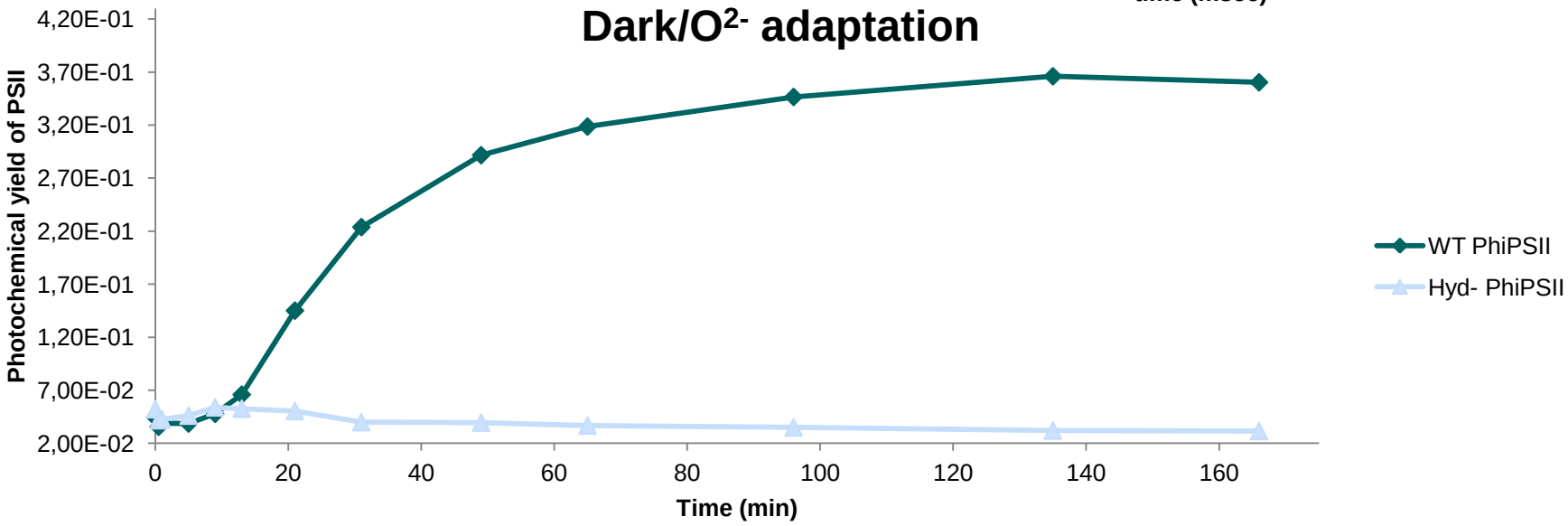




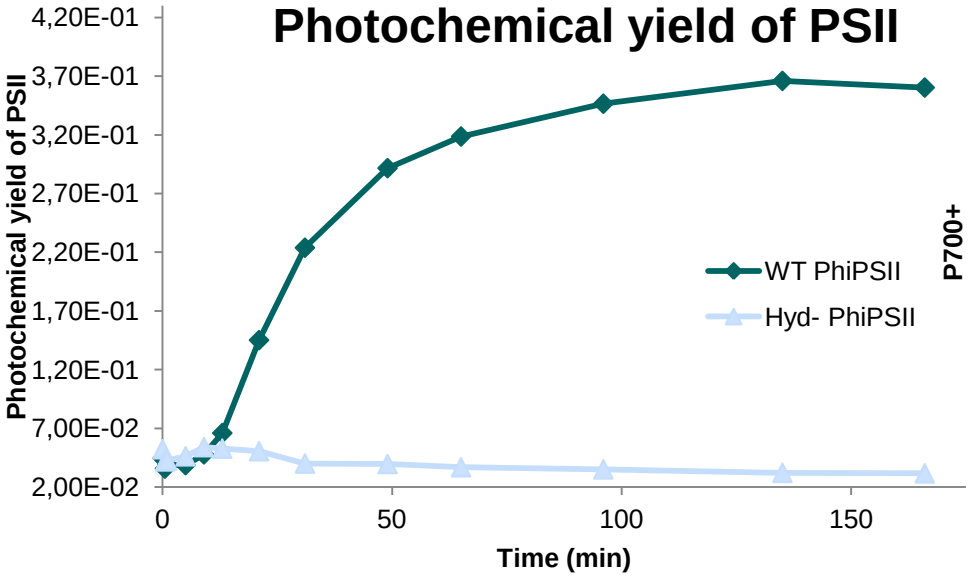
→ hydrogenase is the main electrons acceptor of PSI in anaerobiosis

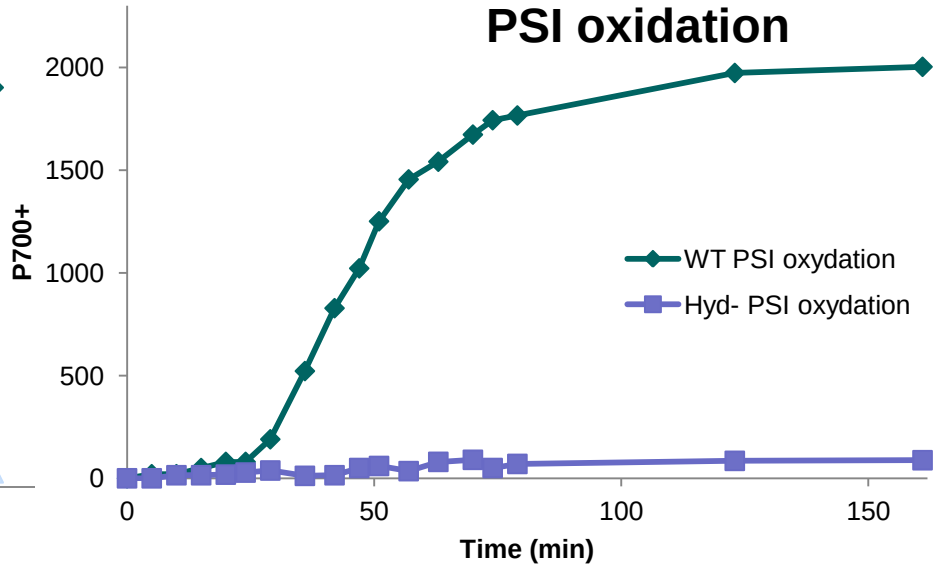
PSII activity (fluorescence emission)

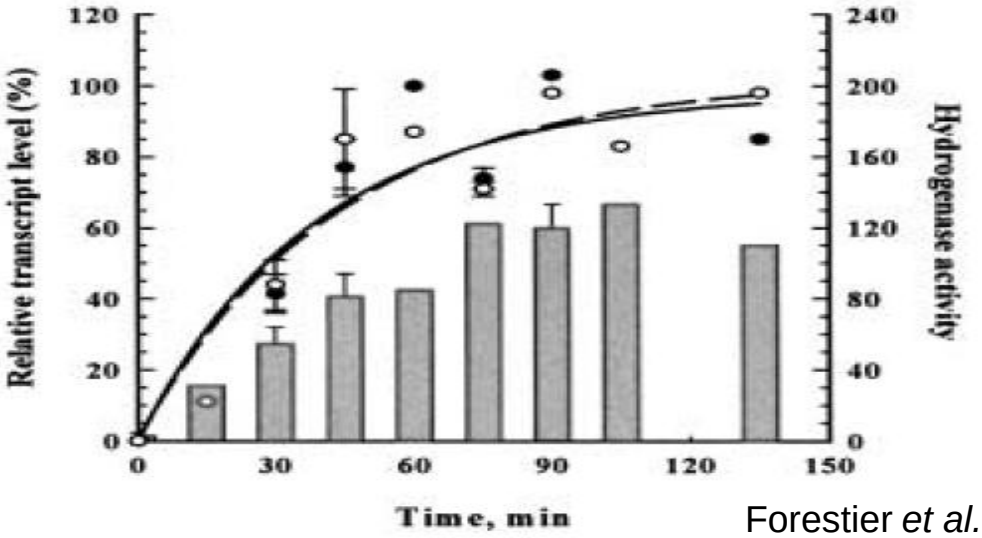




→ Photosynthetic electrons transfer in anaerobiosis is locked in the absence of Hyd

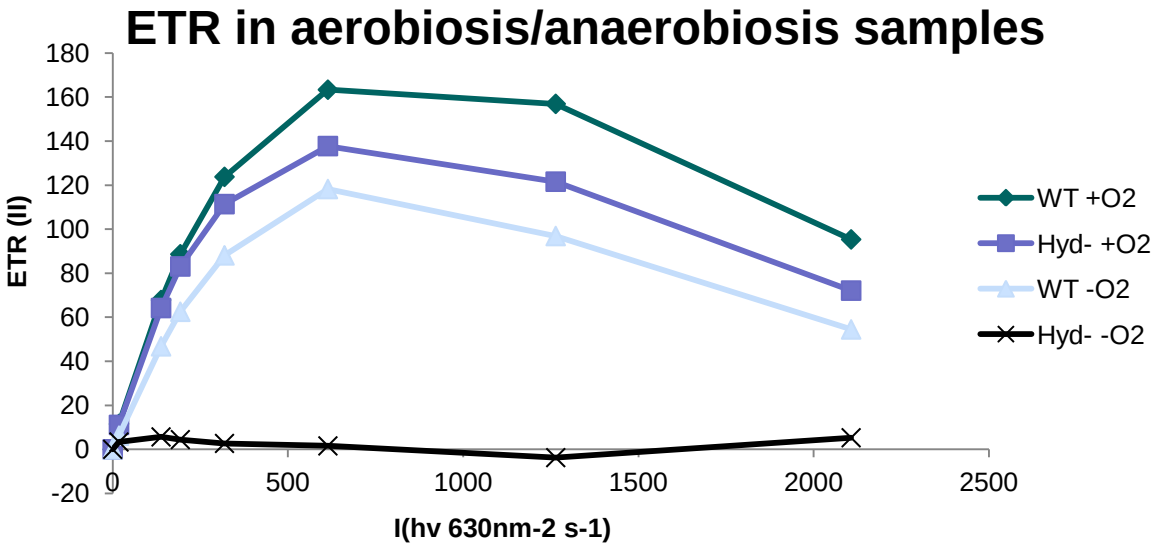






→ PSII, PSI and hydrogenase activities are required for a linear electrons flow in anaerobiosis

Forestier *et al.,* (2003).
Eur. J. Biochem.



Conclusions

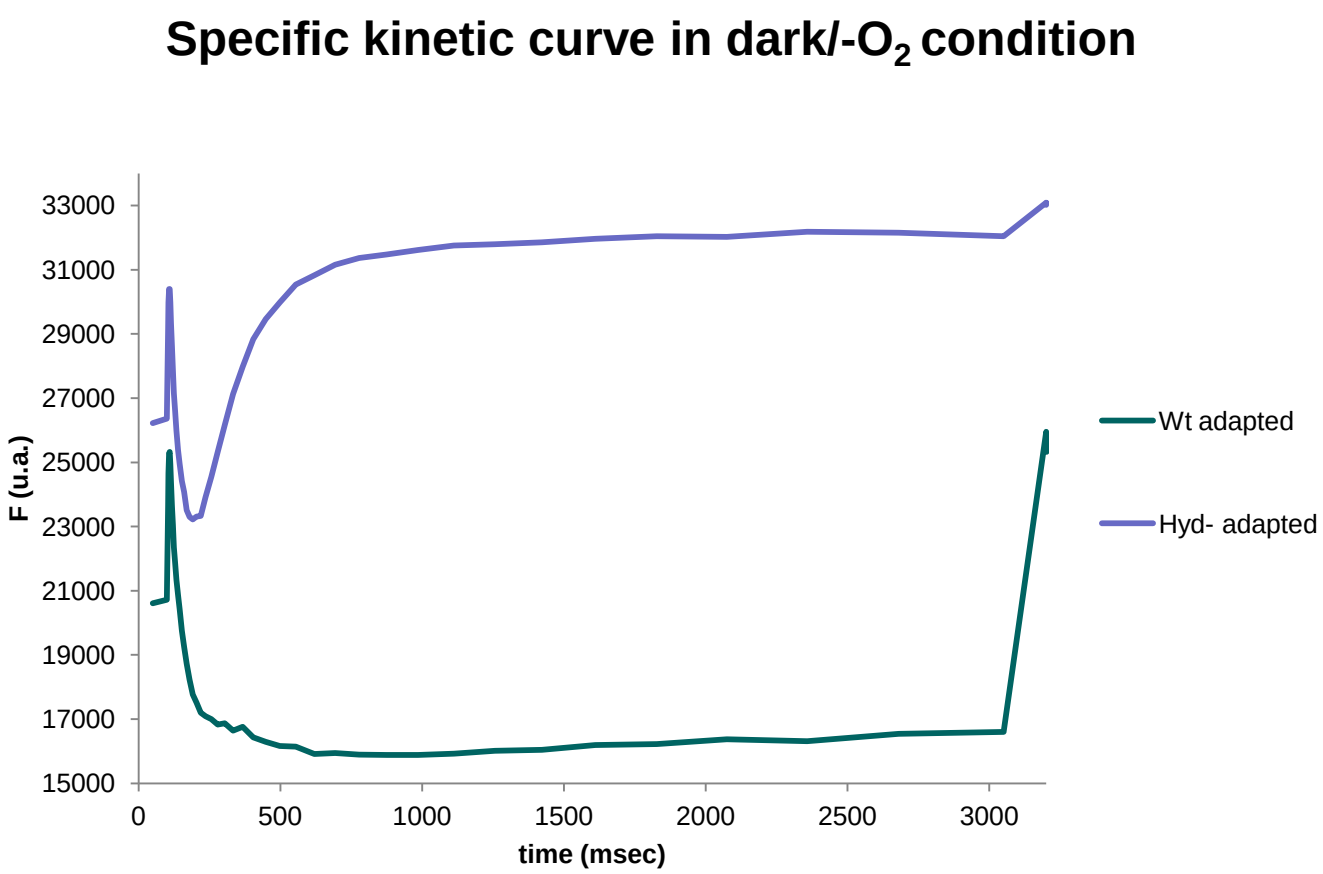
Photosynthetic electrons transfer pathway in anaerobiosis is locked in hydrogenase deficient strain

About 65% of the electrons transfer flow are recovered mainly through the induction of hydrogenase in anaerobiosis in WT strain

Hydrogenase is the main sink for electrons in anaerobiosis

Conclusions

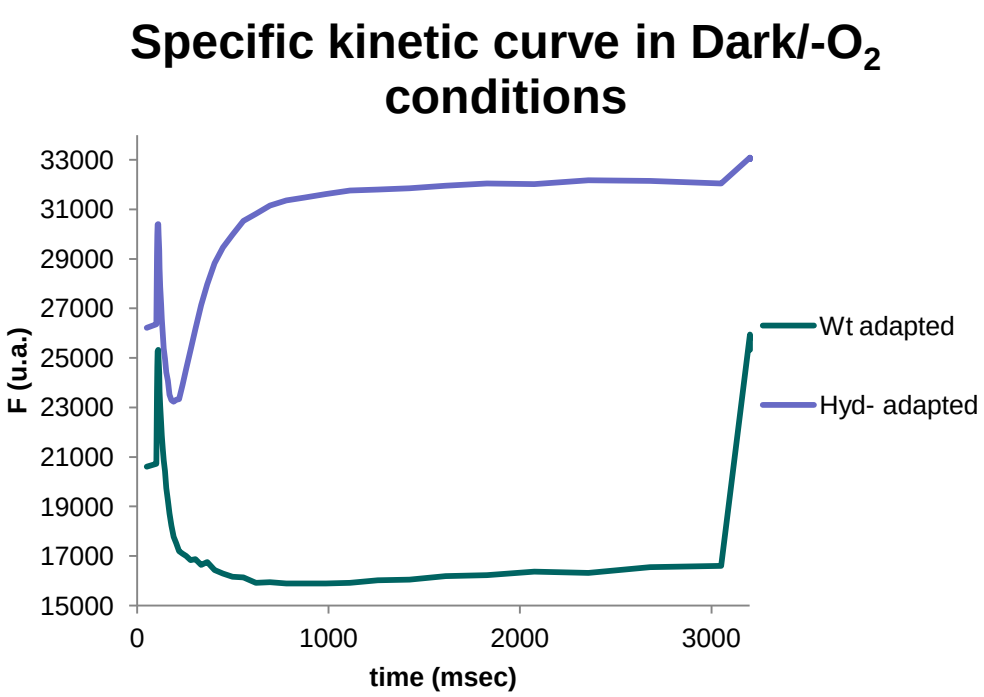
Specific fluorescence signature for a dark/-O_2 adapted hydrogenase deficient strain

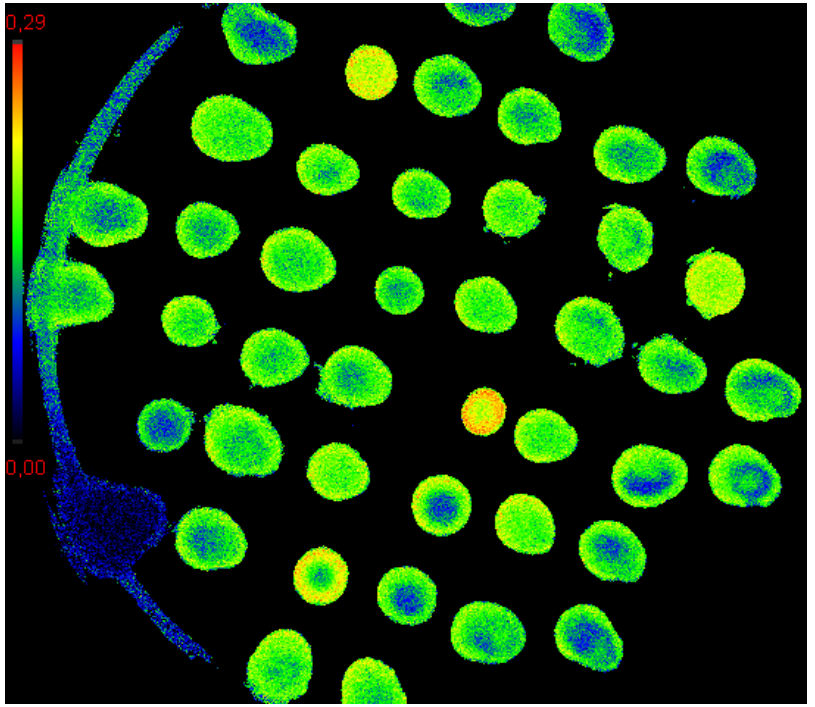


→ It is possible to discriminate deficient mutants for the hydrogenase function

Results: Application to identify new players

A fluorescence camera detects the fluorescence imaging of mutant colonies directly on petri dishes (coll J. Alric (IBPC Paris))

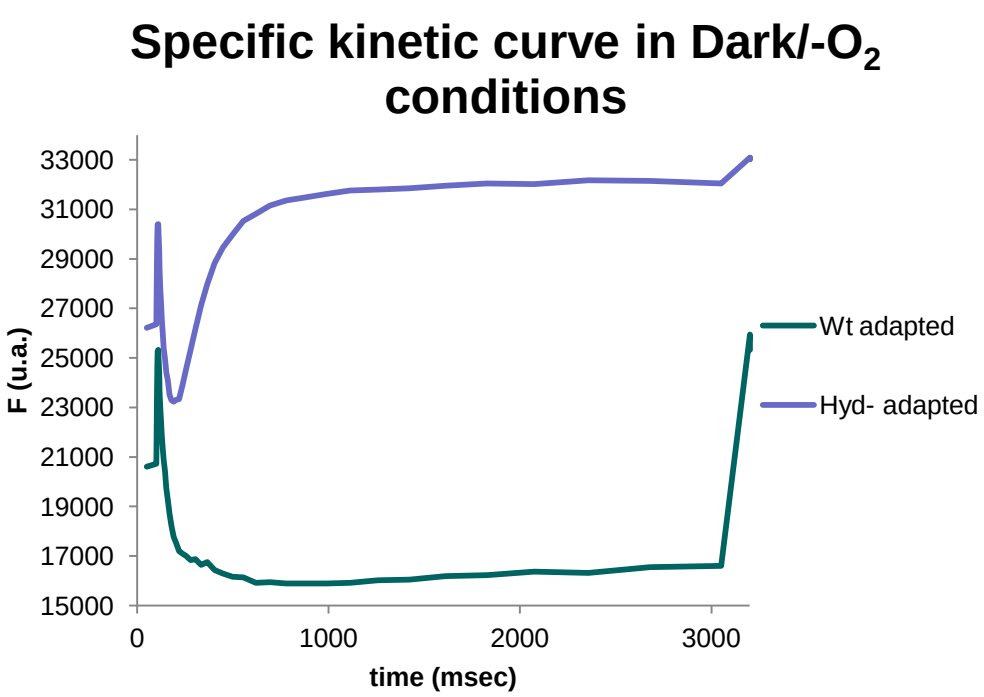


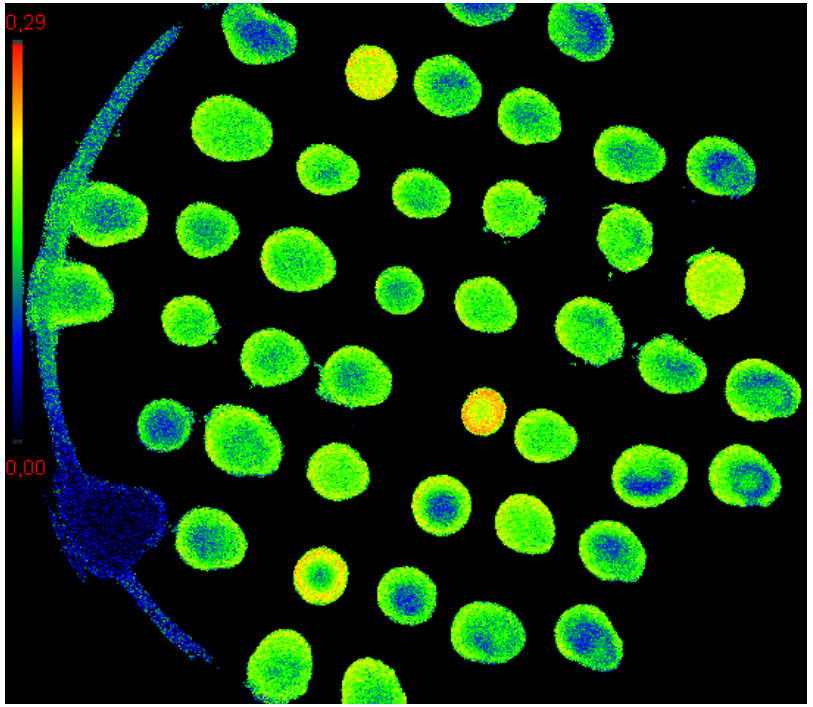


Photochemical yield of PSII (3sec)

Results: Application to identify new players

A fluorescence camera detects the fluorescence imaging of mutant colonies directly on petri dishes (coll J. Alric (IBPC Paris))





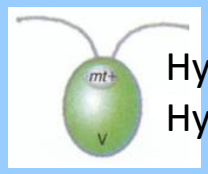
Photochemical yield of PSII (3sec)

→ Screening of 3500 transformants to obtain 30 mutants presenting the specific signature

- Genetics analyses of the hydrogenase deficient strain

Cosegregation analyse →

cosegregation between Hyd$^-$ et Hyg^R



Hyd$^-$
Hyg^R

x

Hyd
Hyg^S

No cosegregation :
No link between Hyd$^-$ et Hyg^R
→ 25% Hyd$^-$ /Hyg^R et 25% Hyd /Hyg^S
25 % Hyd$^-$ / Hyg^S et 25% Hyd /Hyg^R

Cosegregation :
Link between Hyd$^-$ and Hyg^R
→ 50% Hyd$^-$ /Hyg^R et 50% Hyd /Hyg^S

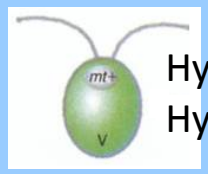
352 descendants analysed			
59% Hyg^R		41% Hyg^S	
100% Hyd$^-$	0% Hyd	0% Hyd$^-$	100% Hyd

→ Cosegregation between hygromycin resistance cassette and hydrogenase deficiency

- Genetics analyses of the hydrogenase deficient strain

Cosegregation analyse →

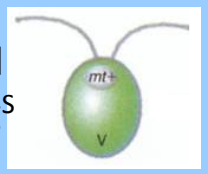
cosegregation between Hyd⁻ et Hyg^R



Hyd⁻
Hyg^R

x

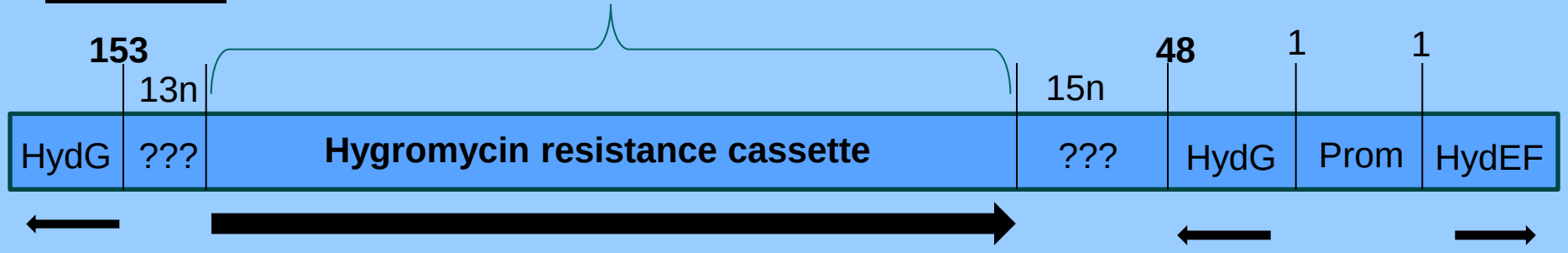
Hyd
Hyg^S



No cosegregation :
No link between Hyd⁻ et Hyg^R
→ 25% Hyd⁻ /Hyg^R et 25% Hyd /Hyg^S
25 % Hyd⁻ / Hyg^S et 25% Hyd /Hyg^R

Cosegregation :
Link between Hyd⁻ and Hyg^R
→ 50% Hyd⁻ /Hyg^R et 50% Hyd /Hyg^S

TAIL-PCR → identification of the insertion site



→ Validation of the screening protocole to identify new players in hydrogenase regulation process

Conclusions

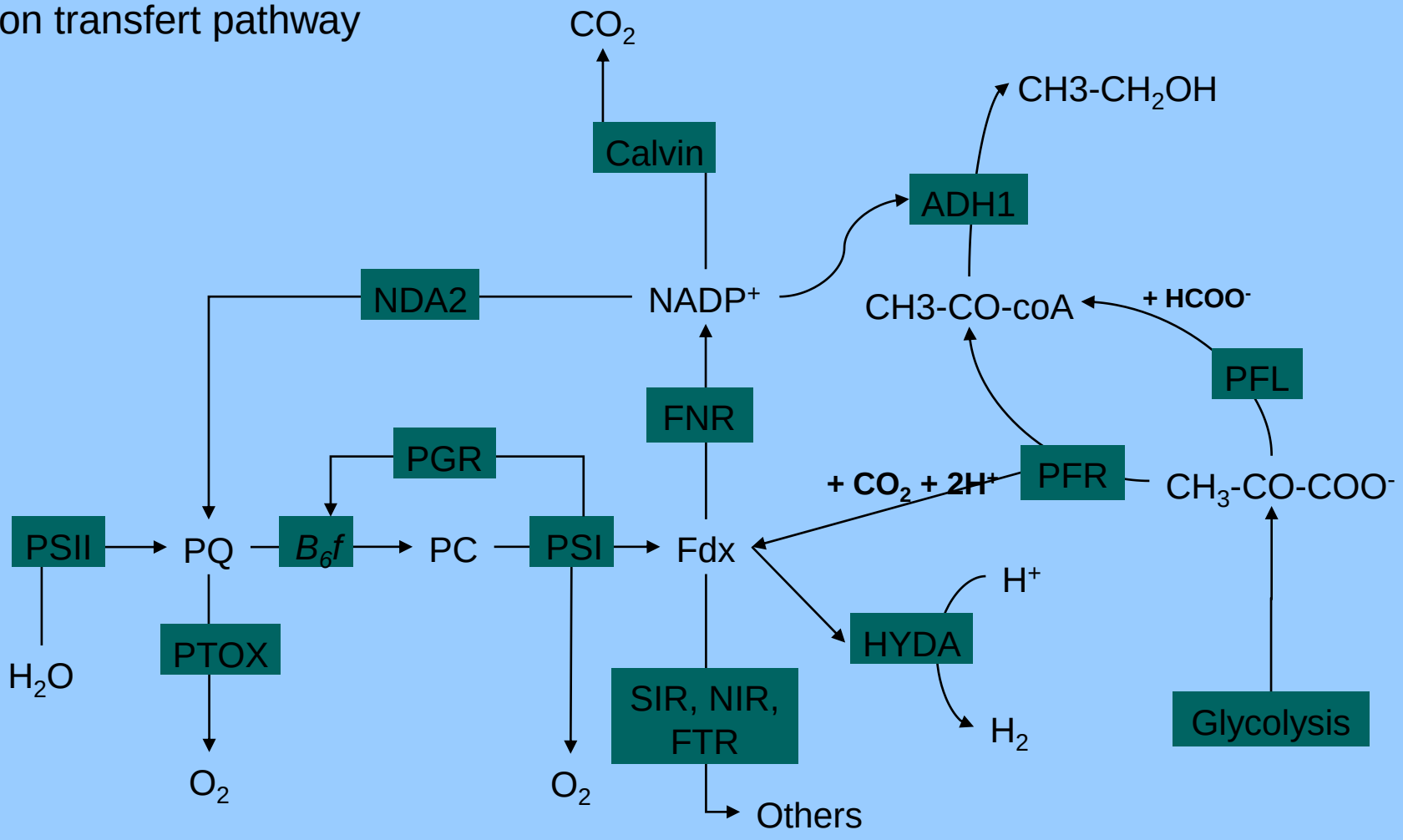
1) Hydrogenase is the main sink for electrons in anaerobiosis. Photosynthetic electron transfer pathway is locked in hydrogenase deficient strain

2) Specific fluorescence signature for a dark/-O_2 adapted hydrogenase deficient strain

3) Development of a new and powerful screening protocol to identify new players in hydrogenase regulation process. Some candidates have already been isolated and are currently characterised.

Outlook

→ Extend the dark/O2- to light transistion study to other mutants of the photosynthetic electron transfert pathway



→ Analyse the candiates already obtained to identify new player in hydrogenase expression, activation or regulation